

Cancer Biology Graduate Program

Part I Qualifying Exam

June 2, 2017

Please use the paper provided to write out your answers. You must answer 8 of the 10 questions.

To pass you must score 70% overall and 7 or higher on 6 questions.

Write the question number at the top left, and your student number on the top right of the first page of each answer.

Good Luck!

Question 1:

You have accepted a position as a postdoctoral researcher in the Department of Cell Biology at Emory University. During the first conversation with your mentor you discuss the work of a former postdoc in the lab who carried out a genetic mosaic screen in the *Drosophila* eye for genes that restrict tissue growth – the goal of which was to identify novel conserved tumor suppressor genes. The former postdoc isolated alleles of a gene she named *slaughterhouse-five* (*sfv*) which has a well-conserved human ortholog. You do not have any experience with fruit flies, but carried out your graduate research using mammalian cell culture and genetic mouse models. You become obsessed with *sfv* and decide to pursue its potential role in mammalian tumorigenesis. A quick search of the literature (published prior to large-scale tumor sequencing efforts) reveals that human *SFV* is contained within a chromosomal region (3p21.3) that exhibits loss-of-heterozygosity (LOH) in human lung cancer cell lines.

- a) Your postdoc position doesn't officially start until after the holidays and you are 'stuck' at your parent's house (bummer) with only a laptop and internet connection. What can you do during this time to start an investigation of whether human *SFV* may be the tumor-suppressor culprit of 3p21.3 in lung cancer? (2.5 points)

b) It's now officially January and you are getting settled into your new laboratory. A colleague down the hall has a lung cancer cell line that forms colonies in soft agar. He has agreed to provide you with cells to do a quick 'proof-of-principle' experiment. Design a quick in vitro experiment to test your hypothesis that human SFV acts as a tumor suppressor. (2.5 points)

c) Based on your investigation in question (a) you hypothesize that loss of a single copy of *SFV* (haploinsufficiency) predisposes to lung cancer. Design an experiment to test your hypothesis *in vivo*. (2.5 points)

- d) While carrying out your experiment in (c) you discover that homozygous loss of *Sfv* is lethal. Moreover, you have extended your analysis in (a) and discovered that LOH of SFV commonly co-occurs with oncogenic KRAS mutations in lung cancer. You hypothesize that loss of SFV promotes the progression of KRAS-mutant lung cancers. Design an experiment to test your hypothesis *in vivo*. (2.5 points)

Question 2:

a) Describe a cell type that may inhibit the ability of effector (CD8+) T lymphocytes to eliminate a tumor in the host (2 points).

b) Name one mechanism by which this 'suppressive' cell may limit CD8+ T cell activity against tumors (2 points)

c) Design an animal experiment describing how you might enhance the ability of CD8+ T cells to elicit anti-tumor immune response by interfering with the suppressive cells or mechanisms they use to limit immunity. Please describe the following:

i. experimental and control groups (2 points)

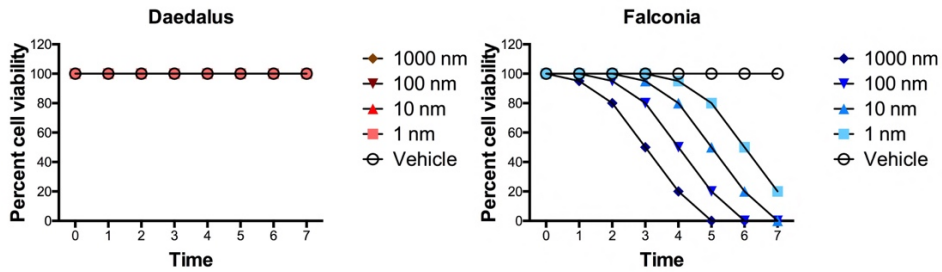
ii. characteristics of the choice of animal and tumor used (2 points)

iii. what biomarkers/experimental readouts in the animals during the study or in their tissues post-mortem would be useful (1 point)

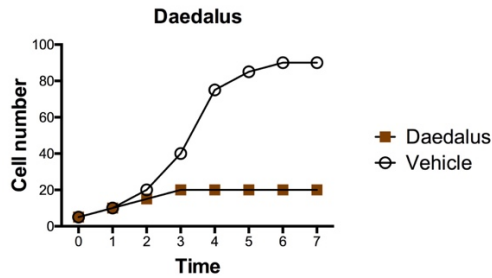
iv. expected results from the experiment (1 point)

Question 3:

You've discovered two drugs that have cytotoxic effects on cancer cells, Daedalus and Falconia. To test how efficiently they kill cells, you treated cancer cells with increasing concentrations of either drug and tested their effects on cell viability over time. Percent cell viability, as determined by an MTT assay, in the presence of drug is shown below. MTT assay assess cell metabolic activity.

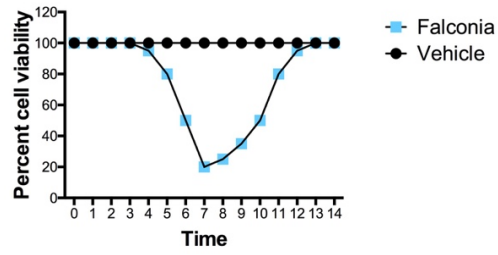


a) While assessing cell viability you noticed that there are fewer cells in your Daedalus-treated samples, so you plotted total cell number over time and obtained a plot shown below. How do you explain the results from your two experiments with Daedalus? Describe two ways to test your hypothesis of what Daedalus is doing to the cells. (6 points)



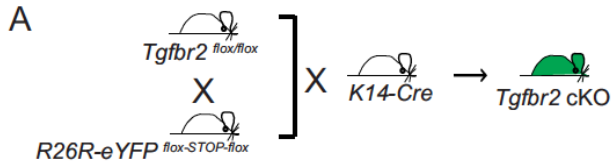
b) *Falconia* appears to have detrimental effects on cell viability. Describe how you would test if cells are dying by apoptosis or necroptosis. (2 points)

c) You tested the long-term effects of Falconia treatment on your cells and discovered an interesting phenomenon. What do you think is going on? Describe one way to test your idea. (2 points)



Question 4:

Below is an excerpt and figure from a recent paper:



In this study, we used the murine *Tgfb2*-deficient anorectal squamous cell carcinoma (SCC) model to study the consequences of loss of TGF β signaling in cancer stem cell (CSC)-driven tumor propagation and metastasis. We found that these *Tgfb2* conditional knockout (cKO) anorectal SCC, which spontaneously metastasize to the lungs, contain a unique population of epithelial cells with features of CSCs, including expression of the CSC marker CD34... We uncovered a novel mechanism linking loss of TGF β signaling with invasion and metastasis via the RAC-activating GEF ELMO1. We show that *Elmo1* is a novel target of TGF β signaling via SMAD3 and that restoration of *Tgfb2* results in complete block of ELMO1 in vivo. ... Taken together, these data demonstrate that upregulation of the GEF ELMO1 is required for *Tgfb2*-deficient SCC CD34⁺ CSCs to metastasize.

- a) Describe the experiments you would perform to isolate CSCs in this model, show that CD34 is a CSC marker, and functionally demonstrate that they are actually CSCs (2.5 points)

b) Given the fact that there was no previous data linking TGF β and ELMO, how do you think the authors uncovered the link between loss of TGF β signaling and the RAC-activating GEF ELMO? What experiments would they do to confirm that this link is real? (2.5 points)

c) Describe the experiments you would perform to support the statement: "We show that Elmo1 is a novel target of TGF β signaling via SMAD3 and that restoration of Tgfbr2 results in complete block of ELMO1 in vivo." (2.5 points)

d) Describe the experiments you would perform to support the statement: "Taken together, these data demonstrate that upregulation of the GEF ELMO1 is required for Tgfr2-deficient SCC CD34⁺ CSCs to metastasize." Why do you think ELMO1 promotes metastasis? (2.5 points)

Question 5:

A pathologist notes that tissue invasion of a tumor seems to be associated with metastasis and poor patient survival.

a) Design a study that would determine if this was an accurate observation. (2.5 pts)

b) The study you designed clearly shows that tissue invasion versus lack of tissue invasion is a risk factor for metastasis and patient survival (i.e. patients with invasive tumors are at a higher risk for death than those whose tumors are not invasive). You would now like to determine the genetic basis for this phenomenon. How would you proceed? (2.5 pts)

c) After you determine the genetic basis for this phenomenon, you would like to convert this into a test that may be applied to patient's tumors that could determine the likelihood of metastasis/death from the tumor. What kind of kinds of tests may be developed for this? (2.5 pts)

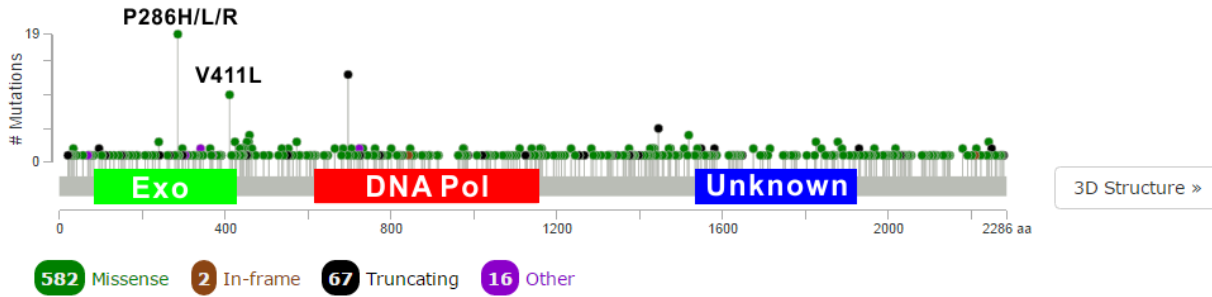
d) What social issues should be considered regarding this test and how could these be addressed? (2.5 pts)

Question 6:

DNA polymerase ϵ (*POLE*) plays a major role in leading-strand DNA synthesis. Its proofreading (exonuclease) function detects and removes misincorporated bases in the daughter strand through failed complementary pairing with the parental strand. A cBioportal search for Cancer Genomics indicated that there are recurrent mutations of *POLE* at codon 286 (P286H/L/R) and 411(V411L) in various tumor types (see picture below).

POLE:

DPOE1_HUMAN



a) Based on figure above, what is your prediction of the functional consequences of these two mutations? (4 points)

b) What is the likely classification of these tumors and how could one test if this were true? (3 points)

c) What type of treatment will you recommend to patients with these types of tumors and why? (3 points)

Question 7:

Anoikis is a form of programmed cell death resulting from loss of cell and extracellular membrane interaction. Cancer cells must develop anoikis resistance in order to survive in the circulation before forming metastatic foci in distant organs. Protein kinases have been implicated in mediating pro-metastatic signaling in human cancers. Through a high-throughput anoikis assay using human CRISPR sgRNA library targeting the human kinome, you identified a kinase called 'Trk', which may be essential for anti-anoikis property in head and neck cancer. Knockout of Trk significantly sensitized the head and neck cancer cell lines to detachment-induced cell death *in vitro*.

a) Design an experiment to test whether Trk contributes to metastatic process *in vivo*. Use all existing metastasis models, and explain the advantages and disadvantages of the model you have chosen. (3 points)

b) How would you test whether the kinase activity of Trk is required for cancer cells to confer anti-apoptosis and pro-metastatic potential *in vitro* and *in vivo*? Be sure to include appropriate controls necessary to make a firm conclusion. (2 points)

c) You have access to 100 primary tumor tissue samples from head and neck cancer patients and 100 paired lymph node metastasized tumor tissue samples from the same patients. The tissues are paraffin-embedded formalin fixed. If the experiments you proposed above revealed that Trk promotes anti-apoptosis and pro-metastatic signaling in head and neck cancer, what approaches would you do to validate that your finding is clinically relevant using the patient tissues? (2 points)

d) Trk inhibitor LOXO-101 is commercially available. Describe how you would test whether Trk is a novel promising therapeutic target for treatment of metastatic head and neck cancer. How would you determine that LOXO-101 affects anoikis resistance and tumor metastasis by targeting Trk with no off-target effect? (3 points)

Question 8:

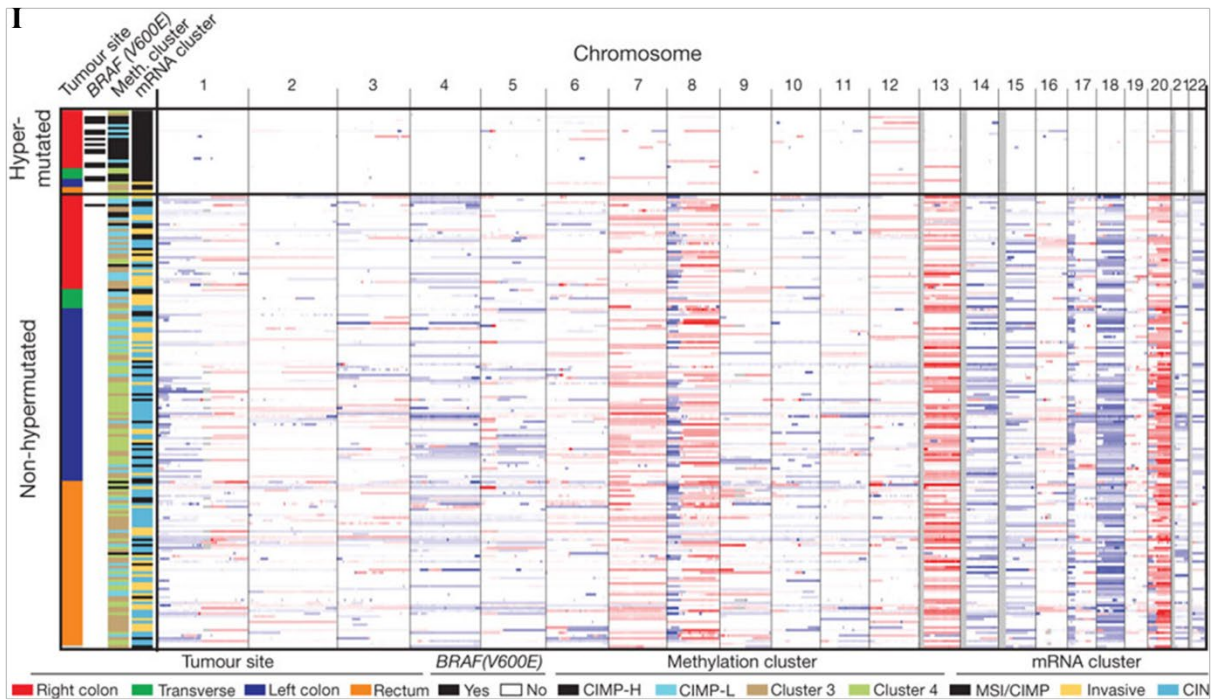
The lab is studying mechanisms involved in the DNA damage response after irradiation of brain tumor cells. Brain tumor cells that can repair their DNA after irradiation survive and cause regrowth of the tumor, inevitably leading to death of the patient. A student in the lab has identified a novel scaffolding protein, BTSP1, that localizes to sites of DNA damage shortly after irradiation in cultured glioma cells. BTSP1 is very highly expressed in glioma cells bearing stem-like properties, which are the drivers of tumor recurrence. He has a great antibody to this protein as well as expression constructs carrying wild type or dominant negative BTSP1. The lab also has a transgenic mouse model of glioma that expresses BTSP1.

a) What experiment might the student have done to lead to this observation? (2 pts)

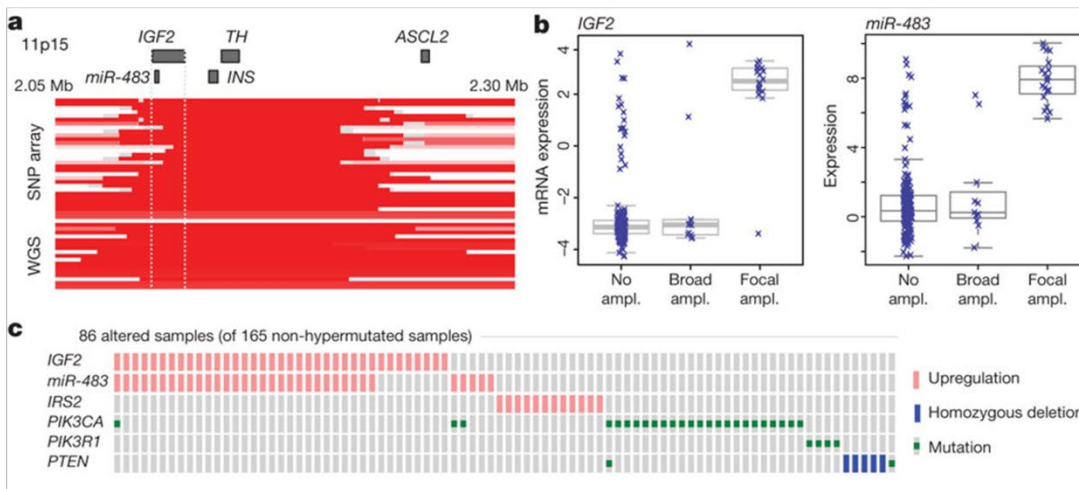
b) How can the student determine whether BTSP1 plays a positive or negative role in DNA repair after irradiation? (3 pts)

c) Design an *in vivo* experiment to determine whether targeting BTSP1 may be of potential therapeutic benefit in glioma.(5 pts).

Question 9:



II



The Figure above is derived from a paper that characterized somatic alterations in 276 colon and rectal carcinomas using a variety of whole genome methods, including exome sequencing, SNP copy number alterations, DNA methylation arrays, mRNA expression and microRNA expression. Tumors were then grouped based on gene expression (mRNA cluster) or DNA methylation (methylation cluster)

Part I summarizes the copy number changes (red=gain, blue=loss) across the 22 chromosomes (p→q arms) compared with 4 other features (left columns: tumor site location; BRAF mutation status (yes/no); methylation ‘class’ (CIMP-H; CIMP-L, 3, 4); and mRNA expression ‘class’ (MSI/CIMP, invasive, CIN) from 195 tumors. Color key for each feature is shown below the Figure. CIMP= CpG island methylator phenotype; MSI= microsatellite instability phenotype.

A subset of these samples (n=97) also underwent low-depth-of-coverage whole-genome sequencing (WGS). A subset of tumors exhibit a 'hypermuted' (> 12 non-silent point mutations per 10^6 bp) phenotype.

a) What are the striking differences between the hypermutated and non-hypermutated tumors with regards to genomic abnormalities and what does this suggest about the mechanisms giving rise to each class? What molecular mechanism might give rise to a hypermutated tumor? (3 points)

b) Chromosome 8 exhibits considerable copy number alteration across the majority of tumors. Would you conclude that the 8p harbors a tumor suppressor gene or an oncogene? What about 8q? Briefly explain your answer. (1 points)

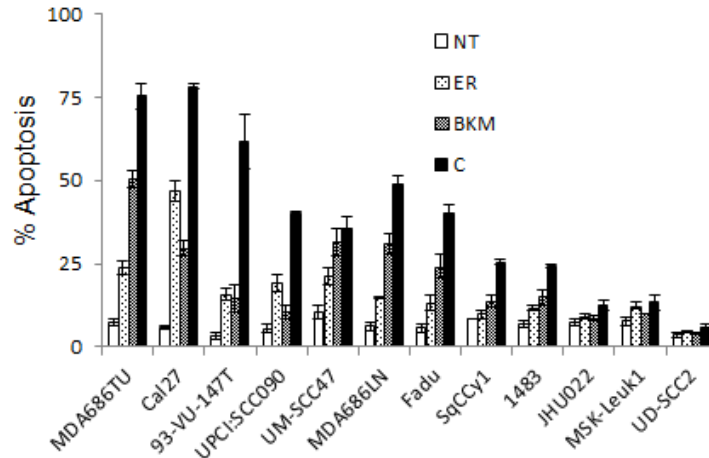
Parts II-a and II-b summarize copy number and expression data across a 0.25 Mb region of chromosome 11p15.5 in a subset of tumors exhibiting focal copy number alterations in this region.

c) What can you conclude about the copy number changes in this region and the resulting molecular alterations to the IGF2 and miR-483 genes found in this subset of tumors? Contrast the two types of assays used to generate the copy number data in (II-a). What is the advantage/ disadvantage of each? (3 points)

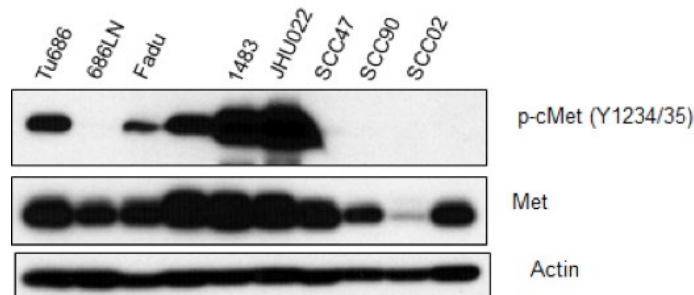
d) Part II-c summarizes molecular alterations in IGF2, IRS2, PIK3CA, PIK3R1, and PTEN found in the non-hypermethylated colorectal cancers. What is interesting about the pattern of changes in these and what does it imply about the functional relationship between the gene products? (3 Points)

Question 10:

Aberrant expression of receptor tyrosine kinases is often associated with drug resistance and treatment failure. After joining to Dr. Smith's lab as a graduate student, he discussed with you that his lab is interested in exploring the mechanism of apoptosis resistance. He shared two pieces of data from his lab shown below. Fig. A represents % apoptosis of 12 head and neck cancer cell lines after treatment with erlotinib (EGFR inhibitor), BKM120 (PI3K inhibitor) or their combination. Although some cell lines are sensitive to apoptosis, others are resistant. Fig. B represents expression of p-Met (active Met), total Met and actin.



A: Induction of apoptosis by erlotinib (ER), BKM120 (BKM) and their combination (C). NT=no treatment



B. Expression of p-Met, Met and actin in a panel of head and neck cancer cell lines

a) Based on Panel B, propose a hypothesis that might explain the resistance of JHU022 cells to the combination of erlotinib and BKM120. Design in vitro experiments using one biochemical and one genetic approach to test your hypothesis. Include all necessary controls to accurately explain your results. What will be the readout for your experiments? (4 points)

b) Design an in vivo experiment to test your hypothesis. Be sure to include controls (4 points)

c) You hypothesize that this mechanism plays a role in a subset of head and cancer patients. How will you test your hypothesis? (2 points)